



<110> Korea Research Institute of Bioscience and Biotechnology

<120> Method for screening of a lipase having improved enzymatic activity using yeast surface display vector and the lipase

<130> 26666U

<150> KR 2002-55575

<151> 2002-09-13

<160> 19

<170> KopatentIn 1.71

<210> 1

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> CALB primer 1

<400> 1

ggctcttcag ccactccttt ggtgaag

27

<210> 2

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> CALB primer 2

<400> 2  
gcggatcctc aggggtgac gat 23

<210> 3  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> CALB primer 3

<400> 3  
gcggatccgg gggtgacgat gccggag 27

<210> 4  
<211> 19  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> GPD-err primer

<400> 4  
gcagagctaa ccaataagg 19

<210> 5  
<211> 19  
<212> DNA  
<213> Artificial Sequence

<220>

<223> T-0 primer

<400> 5

tgcagttgaa cacaaccac

19

<210> 6

<211> 1023

<212> DNA

<213> Candida antarctica

<220>

<221> sig\_peptide

<222> (1)..(51)

<223> secretion signal

<400> 6

atgaatatata ttacatatt tttgttttg ctgtcattcg ttcaaggta cggcactccc

60

ttggtaaagc gtctgccttc cggttccgac cctgcctttt cgcaagccaa gtcggtgctc

120

gatgcggggtc tgacctgcca gggtgcttcg ccattcctcgg tctccaaacc catccttctc

180

gtccccggaa ccggcaccac aggtccacag tcgttcgact cgaactggat ccccccctct

240

gcmcagctgg gttacacacc ctgctggatc tcaccccccgc cgttcatgct caacgacacc

300

caggtaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttcgggcaac

360

aacaagcttc cctgtgtcac ctgggtccag ggtggtctgg ttgcacagtg gggtctgacc

420

ttcttccccca gtatcaggta caaggtcgat cgacttatgg cctttgcgcc cgactacaag

480

ggcaccgtcc tcgccccccc tctcgatgca ctcgcggta gtgcaccctc cgtatggcag

540

caaaccaccc gttcggcaact cactaccgca ctccgaaacg caggtggtct gaccagatc 600  
gtgcccacca ccaacctcta ctcggcgacc gacgagatcg ttcagcctca ggtgtccaac 660  
tcgccactcg actcatccta cctcttcaac gggagaacg tccaggcaca ggctgtgtgt 720  
ggccgcgtgt tcgtcatcga ccatgcaggc tcgctcacct cgcatgtctc ctacgtcg 780  
ggtcgatccg ccctgcgctc caccacgggc caggctcgta gtgcagacta tggcattacc 840  
gactgcaacc ctcttcccgc caatgatctg actccccgagc aaaaggtcgc cgccgctg 900  
ctccccggcgc cggcggctgc agccatcgtg gcgggtccaa agcagaactg cgagcccgac 960  
ctcatgccct acgcccggcc ctttgcatgtt ggcaaaagga cctgctccgg catcgtcacc 1020  
ccc 1023

<210> 7  
<211> 1023  
<212> DNA  
<213> Candida antarctica

<220>  
<221> sig\_peptide  
<222> (1)..(51)  
<223> secretion signal

<400> 7  
atgaatatat ttacatatt tttgttttg ctgtcattcg ttcaaggtag cggcactcct 60  
tttgtgaagc gtctgccttc cggttggac cctgcctttt cgcaagccaa gtcggtgctc 120

gatgcgggtc tgacctgcca aggtgcttcg ccattctcg tctccaaacc catccttctc 180  
gtccccggaa ccggcaccac aggtccacag tcgttcgact cgaactggat ccccctctct 240  
gcgcaagctgg gttacacacc ctgctggatc tcaccccccgc cgttcatgct caacgacacc 300  
caggtcaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttccggcaac 360  
aacaaggcttc ccgtgctcac ctggtcccag ggtggtctgg ttgcacagtg gggctgacc 420  
ttcttccccca gtatcaggta caaggtcgat cgacttatgg cctttgcgcc cgactacaag 480  
ggcacccgtcc tcgccccccc tctcgatgca ctcgcggta gtgcaccctc cgtatggcag 540  
caaaccacccg gttcggcact cactaccgca ctccgaaacg caggtggtct gacccagatc 600  
gtgcccacca ccaacctcta ctcggcgacc gacgagatcg ttcagcctca ggtgtccaac 660  
tcgcccactcg actcatccta cctttcaac ggaaagaacg tccaggcaca ggctgtgtgt 720  
gggccgcagt tcgtcatcgaa ccatgcaggc tcgctcacct cgcatgtctc ctacgtcgta 780  
ggtcgatccg ccctgcgcctc caccacgggc caggctcgta gtgcggacta tggcattacg 840  
gactgcaacc ctcttccccgc caatgatctg actcccgagc aaaaggtcgcc cgccgctcg 900  
ctccccggcgc cggcggtcgc agccatcgta gcgggtccaa agcagaactg cgagcccgac 960  
ctcatgccct acgccccccc ctttgcagta ggcaaaagga cctgctccgg catcgtaacc 1020  
ccc 1023

<210> 8  
<211> 1023  
<212> DNA

<213> Candida antarctica

<220>

<221> sig\_peptide

<222> (1)..(51)

<223> secretion signal

<400> 8

atgaatatata tttacatatt tttgttttg ctgtcattcg ttcaaggta cggcactcct 60

tttgtgaagc gtctgccttc cggttcggac cctgcctttt cgcaagccaa gtcggtgctc 120

gatgcggggtc tgacctgcca gggtgcttcg ccacccctcgg tctccaaacc catccttctc 180

gtccccggaa ccggcaccac aggtccacag tcgttcgact cgaactggat ccccccctct 240

gcmcagctgg gttacacacc ctgctggatc tcaccccccgc cgttcatgct caacgacacc 300

caggtaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttccggcaac 360

aacaagcttc ccgtgctcac ctggtcccag ggtggtctgg ttgcacagtg gggtctgacc 420

ttcttccca gtatcaggta caaggtaatcgat cgacttatgg cctttgcgcc cgactacaag 480

ggcacccgtcc tcgccccccc tctcgatgca ctcgcggta gtgcaccctc cgtatggcag 540

caaaccacccg gttcggcact cactaccgca ctccgaaacg caggtggtct gacccagatc 600

gtgcccacca ccaacctcta ctcggcggacc gacgagatcg ttcagcctca ggtgtccaac 660

tcgcccactcg actcatccta cctcttaaac ggaaagaacg tccaggcaca ggctgtgtgt 720

ggcccgcaatg tcgtcatgca ccatgcaggc tcgctcacct cgcagttctc ctacgtcgtc 780

ggtcgatccg ccctgcgcctc caccacgggc caggctcgta gtgcagacta tggcattacg 840

gactgcaacc ctcttccgc caatgatctg actcccgagc aaaaggctgc cgccggctg 900  
ctcctggcgc cggcggctgc agccatcgtg gcgggtccaa agcagaactg cgagccc 960  
ctcatgccct acgccccccc ctttgagta ggcaaaagga cctgctccgg catcgtcacc 1020  
ccc 1023

<210> 9  
<211> 343  
<212> PRT  
<213> Candida antarctica

<220>  
<221> SIGNAL  
<222> (1)..(17)  
<223> secretion signal

<400> 9  
Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly  
1 5 10 15

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala  
20 25 30

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly  
35 40 45

Ala Ser Pro Ser Ser Val Lys Pro Ile Leu Leu Val Pro Gly Thr  
50 55 60

Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser  
65 70 75 80

Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met  
85 90 95

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr  
100 105 110

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp  
115 120 125

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser  
130 135 140

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys  
145 150 155 160

Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro  
165 170 175

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg  
180 185 190

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser  
195 200 205

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp  
210 215 220

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys  
225 230 235 240

Gly Pro Leu Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe  
245 250 255

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala  
260 265 270

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn  
275                    280                    285

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Pro Ala Pro  
290                    295                    300

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp  
305                    310                    315                    320

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser  
325                    330                    335

Gly Ile Val Thr Pro Gly Ser  
340

<210> 10

<211> 343

<212> PRT

<213> Candida antarctica

<220>

<221> SIGNAL

<222> (1)..(17)

<223> secretion signal

<400> 10

Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly  
1                    5                    10                    15

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala  
20                    25                    30

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly

35                   40                   45  
  
Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr  
50                   55                   60  
  
Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser  
65                   70                   75                   80  
  
Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met  
85                   90                   95  
  
Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr  
100                  105                  110  
  
Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp  
115                  120                  125  
  
Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser  
130                  135                  140  
  
Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys  
145                  150                  155                  160  
  
Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro  
165                  170                  175  
  
Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg  
180                  185                  190  
  
Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser  
195                  200                  205  
  
Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp  
210                  215                  220  
  
Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys

225                  230                  235                  240

Gly Pro Gln Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe  
245                  250                  255

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala  
260                  265                  270

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn  
275                  280                  285

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Pro Ala Pro  
290                  295                  300

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp  
305                  310                  315                  320

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser  
325                  330                  335

Gly Ile Val Thr Pro Gly Ser  
340

<210> 11  
<211> 341  
<212> PRT  
<213> Candida antarctica

<220>  
<221> SIGNAL  
<222> (1)..(24)  
<223> secretion signal

<400> 11

Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly  
1 5 10 15

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala  
20 25 30

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly  
35 40 45

Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr  
50 55 60

Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser  
65 70 75 80

Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met  
85 90 95

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr  
100 105 110

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp  
115 120 125

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser  
130 135 140

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys  
145 150 155 160

Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro  
165 170 175

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg  
180 185 190

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser  
195 200 205

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp  
210 215 220

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys  
225 230 235 240

Gly Pro Gln Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe  
245 250 255

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala  
260 265 270

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn  
275 280 285

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Leu Leu Ala Pro  
290 295 300

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp  
305 310 315 320

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser  
325 330 335

Gly Ile Val Thr Pro  
340

<210> 12

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> CALB primer 4

<400> 12

ctcatatgct acttccggc tcggac

26

<210> 13

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> a-amylase secretion signal

<400> 13

Met Met Val Ala Trp Trp Ser Leu Phe Leu Tyr Gly Leu Gln Val Ala  
1 5 10 15

Ala Pro Ala Leu Ala

20

<210> 14

<211> 317

<212> PRT

<213> Candida antarctica

<400> 14

Leu Pro Ser Gly Ser Asp Pro Ala Phe Ser Gln Pro Lys Ser Val Leu  
1 5 10 15

Asp Ala Gly Leu Thr Cys Gln Gly Ala Ser Pro Ser Ser Val Ser Lys

20

25

30

Pro Ile Leu Leu Val Pro Gly Thr Gly Thr Thr Gly Pro Gln Ser Phe  
35 40 45

Asp Ser Asn Trp Ile Pro Leu Ser Ala Gln Leu Gly Tyr Thr Pro Cys  
50 55 60

Trp Ile Ser Pro Pro Phe Met Leu Asn Asp Thr Gln Val Asn Thr  
65 70 75 80

Glu Tyr Met Val Asn Ala Ile Thr Thr Leu Tyr Ala Gly Ser Gly Asn  
85 90 95

Asn Lys Leu Pro Val Leu Thr Trp Ser Gln Gly Gly Leu Val Ala Gln  
100 105 110

Trp Gly Leu Thr Phe Phe Pro Ser Ile Arg Ser Lys Val Asp Arg Leu  
115 120 125

Met Ala Phe Ala Pro Asp Tyr Lys Gly Thr Val Leu Ala Gly Pro Leu  
130 135 140

Asp Ala Leu Ala Val Ser Ala Pro Ser Val Trp Gln Gln Thr Thr Gly  
145 150 155 160

Ser Ala Leu Thr Thr Ala Leu Arg Asn Ala Gly Gly Leu Thr Gln Ile  
165 170 175

Val Pro Thr Thr Asn Leu Tyr Ser Ala Thr Asp Glu Ile Val Gln Pro  
180 185 190

Gln Val Ser Asn Ser Pro Leu Asp Ser Ser Tyr Leu Phe Asn Gly Lys  
195 200 205

Asn Val Gln Ala Gln Ala Val Cys Gly Pro Leu Phe Val Ile Asp His  
210 215 220

Ala Gly Ser Leu Thr Ser Gln Phe Ser Tyr Val Val Gly Arg Ser Ala  
225                    230                    235                    240

Leu Arg Ser Thr Thr Gly Gln Ala Arg Ser Ala Asp Tyr Gly Ile Thr  
245                    250                    255

Asp Cys Asn Pro Leu Pro Ala Asn Asp Leu Thr Pro Glu Gln Lys Val  
260                    265                    270

Ala Ala Ala Ala Leu Leu Ala Pro Ala Ala Ala Ala Ile Val Ala Gly  
275                    280                    285

Pro Lys Gln Asn Cys Glu Pro Asp Leu Met Pro Tyr Ala Arg Pro Phe  
290                    295                    300

Ala Val Gly Lys Arg Thr Cys Ser Gly Ile Val Thr Pro  
305                    310                    315

<210> 15

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> LQ53 primer

<400> 15

gctgtgtgtg ggccgcagtt cgtcatcg

28

<210> 16

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> LQ35 primer

<400> 16

gcatggtcga tgacgaactg cggcccacac

30

<210> 17

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> LP53 primer

<400> 17

gtcgccgcgg ctgcgctccc ggcgccggcg

30

<210> 18

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> LP35 primer

<400> 18

ctgcagccgc cggcgccggg agcgcagcc

29

<210> 19  
<211> 343  
<212> PRT  
<213> Candida antarctica

<400> 19

Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly  
1 5 10 15

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala  
20 25 30

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly  
35 40 45

Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr  
50 55 60

Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser  
65 70 75 80

Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met  
85 90 95

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr  
100 105 110

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp  
115 120 125

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser  
130 135 140

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys  
145 150 155 160

Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro  
165 170 175

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg  
180 185 190

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser  
195 200 205

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp  
210 215 220

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys  
225 230 235 240

Gly Pro Leu Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe  
245 250 255

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala  
260 265 270

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn  
275 280 285

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Leu Leu Ala Pro  
290 295 300

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp  
305 310 315 320

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser  
325 330 335

Gly Ile Val Thr Pro Gly Ser  
340